

IN THE SPECIFICATION

Please amend the Title on page 1 as follows:

Process for ~~Purification~~ Purifying of Difructose-Dianhydride-III ~~Difructose-Dianhydride III~~

Please replace the paragraph on page 4, line 25, with the following rewritten paragraph:

That is, the present inventors found for the first time as a result of a many-faceted attack on the problem that highly pure DFA III crystals of which the purity reaches 95% or more, specifically 95-99%, can be obtained very efficiently, not by passing a DFA III solution through a column of granular active carbon, but by adding a small amount of powdered active carbon to a DFA III solution, stirring the latter, and applying the latter to filtration with diatomaceous earth filtration and subsequent filtration through a membrane filter, followed by solid-liquid separation, concentration and crystallization of the liquid condensate. It was also found, in the course of this study, that the DFA III fraction obtained by chromatography of a DFA III containing solution can be subjected to direct crystallization after addition of powdered active carbon and subsequent solid-liquid separation and that the crystals thus recovered have no smell.

Please replace the paragraph on page 11, line 23, with the following rewritten paragraph:

It is most preferred that DFA III might be ~~produce-ed~~ produced as a single product from inulin by degradation with an enzyme, but practically other fructose polymers than DFA III are produced. This is a cause of decrease of the purity of an enzymolytic solution. Therefore, it is necessary to choose a starting material which yields least contamination of impurities other than inulin for crystallization of DFA III or an enzyme which yields no

fructose polymers but DFA III. If such a ~~pure solution of~~ DFA III containing solution rich in DFA III is obtained, it would be possible to use it per se as a crude solution of DFA III for defecation and filtration, and crystallization in the invention.

Please replace the paragraph on page 12, line 14, with the following rewritten paragraph:

Inulin is treated with inulin hydrolase, which is then deactivated to give a DFA III solution (enzyme reaction solution). In this operation, it is appropriate to use a starting inulin of which the fructose polymerization degree is 10 or more, preferably 10 to 60, in order to efficiently produce highly pure DFA III crystals. As for an enzyme, the above-mentioned one may optionally be used (preferably, for example, IFT derived from *Arthrobacter* sp. AHU1753 strain (FERM BP-8296)), and enzyme treatment and deactivation may be carried out in a conventional way. Thus, a DFA III solution in which R-Bx (Refractometric Brix) is 10 or more, preferably 15 or more, more preferably 20 or more, and even more preferably 20 to 30, and in which the purity of DFA III is 60% or more, preferably 65% or more, more preferably 70% or more, and even more preferably 70 to 85%, can be obtained. According to a chemical synthesis, a DFA III solution can also be prepared as well. As described above, it is appropriate to use a starting material inulin of which the fructose polymerization degree is 10 or more, though it is also possible to use inulin of which the fructose polymerization degree is, for example, approximately 5, because it can satisfactorily be treated in the invention even though syrup as by-product is increased and the frequency of circulating treatment is increased.

Please replace the paragraph on page 14, line 17, with the following rewritten paragraph:

In the above operation, the solid-liquid separation can be carried out directly without using any filter aid such as diatomaceous earth when the separation is conducted, for example, by filtration with an ultra-filter membrane (UF membrane) or filtration with a membrane filter (MF) or continuous centrifugation. As MF membrane, for example, a ceramic membrane (e.g., trade name: Dahlia; Tsukishima Machine Co.) may be used. According to continuous centrifugation (5,000-25,000 rpm, preferably 8,000-15,000 rpm; 6,500-10,000G, preferably 7,500-9,500G; e.g., 10,000 rpm, 8,200G), crystals of DFA III (crystal size 250-500 μm) can be separated from fine crystals (crystals other than DFA III, mainly tetra- and penta-saccharides; crystal size 100 μm or less); thus, this can be applied to ~~filtration~~ solid-liquid separation of crude crystal syrup or crystal syrup.

Please replace the paragraph on page 18, line 4, with the following rewritten paragraph:

The redissolved solution (a DFA III containing purified solution: R-Bx is 10-60, preferably 20-55, more preferably 40-50; DFA III purity is 90% or more, preferably 90-98%, for example, it reaches about 95-98%) may be used in production of the product of DFA III crystals (purity: 98-99% or more; highly purified crystals with no color and no smell) in the same manner as in the above-mentioned crude crystals, that is, through defecation and filtration, concentration of the filtrate, crystallization of the condensate (a crystal mother liquor for the final product) for a product, separation of the product crystals and crystal syrup, and drying and packaging of the product crystals (DFA III purity is 98-99% or more, highly pure DFA III crystals having no color and smell). The syrup (crystal syrup, crude crystal syrup), if required, after or without ~~filtration~~ solid-liquid separation, may be returned to at

least one of the any purification steps from the DFA III containing solution to the product crystals of DFA III ~~product~~ (the flow as shown in Fig. 1).

Please replace the paragraph on page 21, line 25, with the following rewritten paragraph:

In the invention, a DFA III containing solution (crude crystal mother liquor) of DFA III of which the purity is less than 70% can be crystallized on an industrial scale.

Please replace the paragraph on page 24, line 16, with the following rewritten paragraph:

In the invention, it is possible to use a solution containing highly pure DFA III as well as a solution of less pure DFA III as a DFA III solution as described above, and at least one operation, i.e., treatment with yeast, defecation and filtration or chromato-treatment makes it possible first time to use a less pure DFA III solution (purity 70% or less, possibly 60% or less) as starting material, which has not long been used because of economical or industrial reasons, and to purify DFA III efficiently.

Please replace the paragraph on page 24, line 25, with the following rewritten paragraph:

First, the treatment with yeast may be carried out by bringing a DFA III solution contact with yeast, if required with stirring in an incubation condition, or incubated under aeration. As for yeast, baker's yeast, Japanese sake yeast, beer yeast, wine yeast, and other yeast may optionally be used. It is also possible to use dry yeast, compressed yeast and other various commercially available types of yeast, satisfactorily. Since yeast acts on disaccharides or monosaccharide to degrade or incorporate in the microorganism, the

treatment with yeast is effective in removing disaccharides and/or monosaccharide outside the system.

Please replace the paragraph containing Table 1 on page 40, line 1, with the following rewritten paragraph containing Table 1:

The indication of the above samples as shown in Catalogues is shown in the following table 1.

Table 1.

Sample	a	b	c	d
Chemical structure	GFn	GFn	GFn	GFn
Polymerization range			2-60	10-60
Mean degree of Polymerization		18	10-12	20-25
Production		Enzyme synthesis from sugar	Extract from chicory	Separation of inulin
Polysaccharide content			ca. 70%	100%

GFn:

G: Glucose

F: Fructose

n: number of polymerization

2) Analysis Condition

Column: Dionex, CarboPac PA1, 4x250 mm I.D.

Guard column: Dionex, CarboPac PA1 Guard

Column temperature: room temperature

Eluent: Gradient

	0 min.	100 min.
	Rate (%)	Rate (%)
1N-NaOH	15	15
1M-NaOAc (<u>1M</u> <u>sodium acetate</u>)	0	45
Water	85	40
Curve No.	-	2

Detector: Dionex, Pulsed Electrochemical Detector

Detection mode: Integrated Amperometry

Pulse voltage: E1: +0.05V (400m sec),
 E2: +0.75V (200m sec),
 E3: -0.15V (400m sec)

Flow rate: 1.0 ml/min

Range: 1 μ C

Amount injected: 5 μ l each of 0.1% aqueous solution injected

Cycle of analysis: 120 min

3) Results

Please replace the paragraph on page 44, line 5, with the following rewritten paragraph:

(1) The product of Firm D (200kg) is dissolved in 1000kg of hot water at 80°C, and cooled down to 60°C. To the resulting solution is added IFT 5000 units/kg inulin (prepared in the production of an enzyme as shown below), and the mixture is stirred at 60°C for 24 hours to yield a DFA III solution. The reaction mixture is heated up to 80 °C to deactivate the enzyme. To this deactivated solution is added Taiko Active Charcoal S (Futamura Kagaku Kogyo KK; average particle size 35 microns, less than 147 microns) , and the mixture is stirred at 60 °C for 10 minutes. The mixture was then filtered through diatomaceous earth (Showa Chemical Ind.; Radiolite700). That is, the above diatomaceous was pre-coated on the inside outside of a ceramic tube (Japan Pole KK .: PR-12 type ceramic tube), through which a solution containing active carbon was passed under increased pressure, and the filtrate was recovered outside inside the tube.

Please replace the paragraph on page 48, line 11, with the following rewritten paragraph:

Main Culture: The culture broth (1 L, 10 flasks, 1% pre-culture broth or the main culture broth) prepared in the pre-culture was inoculated on Culture medium 2 under sterile condition. The jar fermenter was operated at 27°C for 17 hours. Aeration: 1 vvm (100 L/min); frequency of stirring: 300 rpm.

Please replace the paragraph on page 48, line 16, with the following rewritten paragraph:

(4) Recover of the cells and others: The culture broth prepared in (3) was separated by a centrifuge into the cells and the supernatant (2000G, 4°C, 20 minutes), and the latter was used as a DFA III enzyme solution. The enzyme solution was adjusted at pH 5.5 with phosphoric acid and stocked at -20°C.

Please replace the paragraph on page 50, line 14, with the following rewritten paragraph:

By 20 expert panelists, crystal DFA III; finely pulverized DFA III and granular DFA III were subjected to a sensory test. The results are shown in Table 4. As shown in Table 4, the finely pulverized DFA III had a stronger sweetness than the crystal DFA III and the former was improved in easiness of dissolution and sharpness of sweetness, affording a good result. The granular DFA III showed approximately the same sweetness as the finely pulverized DFA III, but the former was improved in easiness of dissolution in the mouth and sharpness of sweetness, and totally evaluated highly.

Please replace the paragraph on page 52, line 23, with the following rewritten paragraph:

Main Culture: The culture broth (1 L, 10 flasks, 1% pre-culture broth or the main culture broth) prepared in the pre-culture was inoculated on Culture medium 2 under sterile condition. The jar fermenter was operated at 27°C for 17 hours. Aeration: 1 vvm (150 L/min) ; frequency of stirring: 300 rpm.

Please replace the paragraph on page 53, line 3, with the following rewritten paragraph:

(4) Recover of the cells and others: The culture broth prepared in (3) was separated by a centrifuge into the cells and the supernatant (2000G, 4°C, 20 minutes), and the latter was used as a DFA III enzyme solution. The enzyme solution was adjusted at pH 5.5 with phosphoric acid and stocked at -20°C.

Please replace the paragraph on page 54, line 19, with the following rewritten paragraph:

Accession Number: FERM BP-8296

Indication of Deposit: *Arthrobacter* sp. AHU ~~1752~~ 1753

Name of Depository Institution:

International Patent Organism Depositary,

National Institute of Advanced Industrial Science and Technology

Address of Depository Institution:

AIST Tsukuba Central 6, 1-1, Higashi 1-chrome,

Tsukuba-shi Ibaraki-ken, 305-8566 Japan

Date of Deposition: February 18, 2003.